bilayer and therefore plays an important role in maintaining lipid asymmetry. Leaflet composition is regulated by the active transport of lipids by membrane proteins, while thermal diffusion across a membrane tries to randomize the leaflet composition. We have measured the transbilayer diffusion rates for three different sterols over a wide range of compositions. The sterols studied were all cholesterol analogs; including dihydrocholesterol, ergosterol, a component of fungal cell membranes, and stigmasterol, an unsaturated plant sterol. Temperature was varied to determine its influence on transbilayer diffusion rates. We find that sterol structure does have an influence on the rate at which lipids move between bilayer leaflets. Transbilayer diffusion measurements were made using a sodium dithionite assay to monitor the location of lipid analogues within DMPC/sterol liposomes.

838-Pos Board B717

Cholesterol Flip-flop And Chemical Potential In A Systematic Set Of Lipid Bilayers

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Cholesterol is a necessary component of animal cellular membranes. The concentration of cholesterol varies from 0-5 mol% in the endoplasmic reticulum to 25-40mol% in the plasma membrane. Thermal fluctuations cause cholesterol to move normal to the plane of the bilayer. At the extremes, cholesterol can translocate across the bilayer (flip-flop) and diffuse from the bilayer into water (desorption). We have used atomistic and coarse grained molecular dynamics computer simulations to investigate the partitioning of cholesterol through a systematic set of lipid bilayers. Atomistic simulations provide detailed analysis, while inexpensive coarse grained simulations allow more bilayers to be investigated and longer time scales to be sampled. From the coarse grained simulations, cholesterol flip-flop was directly observed, and the rate matched our estimate from the free energy barrier. We find the rate of cholesterol flip-flop is fast and strongly dependent on the structure of the bilayer. The rate of flip-flop is on the microsecond range in fluid, disordered poly-unsaturated bilayers, and on the second range in rigid, ordered bilayers with high cholesterol content. The chemical potential of cholesterol in the bilayer compared to water is equal to our free energies of desorption. We can infer the relative affinity of cholesterol for the bilayers by comparing the chemical potentials. We find cholesterol prefers more ordered and rigid bilayers with saturated acyl tails, and high cholesterol content. Cholesterol has the lowest affinity for poly-unsaturated lipids.

839-Pos Board B718

The Behavior of Two Oxidized Derivatives of Cholesterol in Model Membranes

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Cholesterol's role in ordering lipid membrane domains is well known. Even subtle changes in the structure of this sterol greatly affect the biophysical dynamics of membranes, usually because of perturbations in the interactions between the sterol and other membrane lipids that chemical modifications cause.

Cholesterol oxidation products (oxysterols), which result from enzymatic and non-enzymatic mechanisms, are cytotoxic and found in atherosclerotic plaques. Previous studies have shown that the membrane properties of oxysterols vary, depending on the specific site of the oxygen-containing moiety. In this study, we examined the interactions of two oxysterols, one formed through non-specific oxidation (7-ketocholesterol), and one produced enzymatically (25-hydroxycholesterol) with two common membrane lipids, 1-palmitoyl-2-oleoyl-*sn*-phosphocholine (POPC) and brain-derived sphingomyelin.

Analysis of force-area isotherms obtained by compression of pure sterol monolayers and of binary monomolecular films at the air-water interface, comprised of varying mole fractions of POPC or sphingomyelin and either oxysterol, reveals significant differences in surface behavior with respect to each other and to native cholesterol. Both oxysterols condensed POPC and sphingomyelin films to a lesser degree than cholesterol, and an expansion of sphingomyelin films was observed with low mole fraction 7-ketocholesterol. Additionally, surface compression moduli data obtained from the force-area isotherms reveal a decreased ability of both oxysterols to mitigate the phase transition of sphingomyelin compared to cholesterol. The changes of membrane behavior in the presence of oxysterols reported here suggest a relation of their toxicity to the propensity of lipids membranes to form liquid-ordered domains (rafts).

840-Pos Board B719

A Calorimetric and Spectroscopic Comparison of the Effects on Ergosterol and Cholesterol on the Thermotropic Phase Behavior and Organization of Dipalmitoylphosphatidylcholine Bilayer Membranes

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We performed comparative DSC and FTIR spectroscopic measurements of the effects of cholesterol (Chol) and ergosterol (Erg) on the thermotropic phase behaviour and organization of DPPC bilayers. Erg is the major sterol in the biological membranes of yeasts, fungi and many protozoa. It differs from Chol in having two additional double bonds, one in the steroid nucleus at C7-8 and another in the alkyl chain at C22-23. Erg also has an additional methyl group in the alkyl chain at C24. Our DSC studies indicate that the incorporation of Erg is more effective than Chol is in reducing the enthalpy of the pretransition. At concentrations below 10 mol%, Erg is also more effective than Chol in reducing the enthalpies of both the sharp and broad components of main phase transition. However, at sterol concentrations from 30-50 mol %, Erg is generally less effective at reducing the enthalpy of the broad components and does not completely abolish the cooperative hydrocarbon chain-melting phase transition at 50 mol% as does Chol. Moreover, in this higher ergosterol concentration range there is no evidence of the formation of ergosterol crystallites or of the lateral phase separation of Erg-enriched phospholipid domains. Our FTIR spectroscopic studies demonstrate that Erg incorporation produces a less tightly packed bilayer than does Chol which is characterized by increased hydration in the glycerol backbone region of the DPPC bilayer. These and other results indicate that Erg is less miscible in DPPC bilayers at higher concentrations than is Chol.

841-Pos Board B720

Schiff Base Formation Between The Cholesterol Oxidation Product 3β-hydroxy-5-oxo-5,6-secocholestan-6-al And Amino Phospholipids

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The keto-aldehyde 3β-hydroxy-5-oxo-5,6- secocholestan-6-al is formed by the oxidation of cholesterol with ozone. This oxidized form of cholesterol is associated with a number of pathological conditions including atherosclerotic plaques, Alzheimer's and Parkinson's diseases. We have shown earlier that the compound can react covalently with the amino group of phosphatidylethanolamine to form a Schiff base . Here, using a spectroscopic technique, we determine the kinetics of the Schiff base formation between 3β-hydroxy-5-oxo-5,6- secocholestan-6-al and dimyristoylphosphatidylethanolamine in both the gel and liquid crystalline states of the phospholipid. The activation energies of this reaction in the two states are also calculated. In addition, we determine that a Schiff base can also be formed with the amino group of phosphatidylserine, albeit with slower kinetics. These findings are significant as they show that oxidized cholesterol can react covalently not only with the amino groups of proteins, but also with the amino groups of phospholipids, potentially influencing the structure of biological membranes.

842-Pos Board B721

A Comparison Of Ceramide And Ceramide-1-phosphate Miscibility In Phosphatidylcholine Bilayers

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Sphingolipids are key lipid regulators of cell viability: ceramide is one of the key molecules in inducing programmed cell death (apoptosis), whereas other sphingolipids, such as ceramide 1-phosphate, are mitogenic. The phase behavior of bilayers comprising binary mistures of N-hexadecanoyl-D-erythro-ceramide (C_{16}-ceramide) or N-hexadecanoyl-D-erythro-ceramide-1-phosphate (C_{16}-ceramide-1-phosphate; C_{16}-C1P) with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were studied using differential scanning calorimetry (DSC) and deuterium nuclear magnetic resonance (^{2}H-NMR). Partial phase diagrams (up to a sphingolipid mole fraction of X=0.40) were constructed for both mixtures. For C_{16}-ceramide-containing bilayers DSC heating scans at X_{cer}=0.025 showed a complex structure of the main phase transition peak suggestive of lateral phase separation. The transition width increased significantly upon increasing X_{cer}, and the upper phase boundary temperature of the mixture shifted to ~65°C at X_{cer} = 0.40. The temperature range over which ^{2}H-NMR spectra of C_{16}-ceramide/DPPC-d_{62} mixtures displayed coexistence of gel and liquid crystalline domains increased from ~10° for X_{cer}=0.1 to ~21° for X_{cer}=0.4. DSC and ^{2}H-NMR observations of C16-C1P/DPPC mixtures at corresponding concentrations indicated that two-phase coexistence was limited to significantly narrower ranges of temperature for mixtures containing C16-C1P

compared to those containing C_{16}-ceramide. Work supported by funding from the Sigrid Juselius Foundation (JMH), the Finnish Cultural Foundation (JMH), Evald and Hilda Nissi Foundation (JMH), The Finnish Eye Foundation (JMH), the Academy of Finland (AH, IV, SW), and from the Natural Sciences and Engineering Research Council of Canada (MRM).

843-Pos Board B722

Sterol Solubility in Vesicles (GUV's) Containing the Ternary Lipid Mixture DPPC:DOPC:Sterol by Quantitative NMR

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In the laboratory, giant unilamellar vesicles (GUVs) offer a rich system for studying miscibility of cholesterol and phospholipids in a lipid bilayer. Our laboratory produces vesicles through various methods, but this study will focus on electroformation. Our work and the work of many other laboratories rely on the assumption that the electroformation process creates vesicles with the same lipid composition originally assembled in the electroformation chamber, up to an ultimate sterol solubility limit. A few sterol solubility limits have been previously tested in selected binary systems [1], but are not generally known for ternary systems. It is important for us to test our assumption that the electroformation process does not significantly alter lipid composition, and also to determine the solubility limit of sterols in membranes in order to understand and properly present the results of other GUV studies from our laboratory. Here we describe studies using quantitative NMR to ascertain the solubility of sterols for which convenient chemical assays exist (e.g. cholesterol) and for which chemicals assays are not readily available (e.g. ergosterol).

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844-Pos Board B723

Modeling The Temperature Dependence of Membrane Solubilization by Detergents

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It is known that lipid membranes become, typically, less susceptible to solubilization with increasing temperature so that more detergent is needed to start and complete their conversion to mixed micelles. Qualitatively, this can be explained by the fact that thermal chain disordering and headgroup dehydration render the spontaneous curvature of the molecules less positive.

Here we present an alternative model to describe this temperature dependence quantitatively in terms of simple, physically meaningful model parameters. This model relates the onset of solubilization to the detergent concentration when micellization becomes more favorable than membrane insertion. It quantifies the effect of temperature on membrane versus micellar packing effects in terms of the heat capacity changes of partitioning (-0.75 kJ/(mol K)) being more negative than that of micelle formation (-0.52 kJ/(mol K)). We demonstrate the model based on measurements of the CMC, partition coefficient and heat capacity changes for pentaethylene glycol monodecyl ether (C10E5) interacting with membranes of POPC by isothermal titration calorimetry (ITC).

845-Pos Board B724

An Approximate Cooperativity Analysis By Dsc And Uv-vis Phase Of Pseudobinary DXPC-DC8,9PC-Cholesterol Dispersions

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The effect of DC8,9PC on the cooperativity of ordered-fluid phospholipid phase transitions has been investigated by determining the transitions widths in multilayer's dispersions of DXPC - DC8,9PC by DSC. The van' t Hoff values of the main transition enthalpies were calculated using an approximate expression deduced from Zimm and Bragg theory. The DC8,9PC decreases the cooperativity (size of the cooperative unit of synthetic DXPC bilayers). The observation that the membrane lipids in the mixed lipid/DC8,9PC assemblies appear to adopt bilayer structures is important, since it demonstrates that the lipid domains within the colorimetric vesicles exist in the fundamental organizational unit found in cellular membranes. The experiments described in this work shed light upon the effects of external environmental parameters, such as temperature, upon structural and dynamical properties of the organized lipid assemblies. The colorimetric platform also facilitates elucidation of the contribution of distinct membrane components, such as cholesterol, toward shaping membrane properties. This capability could open the way for application of the assay for detailed analyses of the roles played by particular molecules, such as peptides and DNA, in determining membrane functions and properties. Between non polymeric and polymeric species in DXPC: DC8,9PC mixtures the influence of the polymer is from C14 chain-down more cooperative the non-polymeric; from C16 on before DPPC:DC8,9PC is better polymerized than non-polymerized and for DSPC:DC8,9PC there is no difference. Hypothesis is C14 or less non-polymerized are intermixed with no difference but when polymerized, non ideal mixing is formed, polymers containing polymeric units with pockets containing DMPC and/or cholesterol. When chains are C16 or more units of saturated and non saturated mixtures cooperative units are quite similar.

846-Pos Board B725

Energetics of Cholesterol Transfer between Lipid Bilayers

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It is believed that natural biological membranes contain domains of liquid ordered phase enriched in cholesterol and sphingomyelin. Although the existence of these domains, called lipid rafts, is still no firmly established for natural membranes, direct microscopic observations and phase diagrams obtained from the study of three-component mixtures containing saturated phospholipids, unsaturated phospholipids, and cholesterol demonstrate the existence of lipid rafts in synthetic membranes. The presence of the domains or rafts in these membranes is often ascribed to the preferential interactions between cholesterol and saturated phospholipids, for example, between cholesterol and sphingomyelin. We calculated, using molecular dynamics computer simulation technique combined with the umbrella sampling and weighted histogram analysis method (WHAM), the free energy of cholesterol transfer from the bilayer containing unsaturated phosphatidylcholine lipid molecules to the bilayer containing sphingomyelin molecules and find that the affinity of cholesterol to sphingomyelin is higher. By doing the simulations at different temperatures, we calculated the free-energy components, energy and entropy, and show that cholesterol transfer is exothermic and with a loss of entropy. The transfer is promoted by the favorable change in the lipid-lipid interactions near cholesterol and not by the favorable energy of cholesterol-sphingomyelin interaction, as assumed previously.

847-Pos Board B726

Impact Of Ceramide3 On POPC Host Membranes: A Study On Structure And Thermodynamics

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The uppermost layer of skin epidermis exhibits a very peculiar lipid composition consisting mostly of long-chain ceramides of asymmetric chain length. The functional impact of ceramides on 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membranes was investigated using natural phytosphingosine type ceramide3 (Cer3) as a model system. Results will be presented on structure and thermodynamic phase behavior of the composite model system for three mole ratios Cer3/POPC (5:95; 10:90; 15:85). All methods applied so far (SAXS; DSC; confocal microscopy) reveal non-ideal miscibility of the two compounds with macroscopic separation of coexisting lamellar phases of different rigidity. Two major transition regions were identified: at low temperature(T_{m1}) and at high temperature(T_{m2}). They were attributed to POPC rich and Cer3 rich domains respectively. A slight increase in the d-spacing and as well a shift of T_{m1} towards higher values indicate a solidifying effect of Cer3 on POPC host membranes. At low concentration the Cer3 rich domain (T_{m2}) exhibits normal thermodynamic behavior with freezing point depression due to the presence of POPC. The continuous shift in T_{m1} together with a loss of transition cooperativity for the POPC rich domain hints towards a modification of the bilayer elasticity due to the presence of the Cer3. The overall heat content of the composite system increases with the amount of Cer3 present, signifying a stronger attractive interaction among the lipids. The Cer3 rich phase is almost dehydrated. Giant vesicles (GUVs) exhibit fluid-gel domain coexistence with the typical smooth and flat face facets as signatures of the more fluid and the more rigid membrane regions.

848-Pos Board B727

The Effect Of Glycerol On Membrane Solubilization By Nonionic Surfactants

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Some protocols for solubilizing membrane components by surfactants use glycerol as a co-solute for stabilizing the native state of proteins. We have studied